External Peer Review

IRIS Toxicological Review for n-Hexane

Final Report

Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Prepared by
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April 2005

This document was prepared for the EPA by ORISE under interagency agreement
No. DW-89939822-01-0 between EPA and the U.S. Department of Energy.
ORISE is managed by Oak Ridge Associated Universities under a contract with DOE.

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IRIS Toxicological Review for n-Hexane

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Charge to External Reviewers for the IRIS Toxicological Review for n-Hexane

The U.S. EPA is conducting a peer review of the scientific basis supporting the human health risk assessment of n-hexane that will appear on the Agency's online database, the Integrated Risk Information System (IRIS).

The draft documents for the external peer review contain a description of the oral database, an inhalation reference concentration, and a qualitative cancer assessment. Please provide detailed responses to the charge questions below.

Charge questions:

1) Oral reference dose (RfD) for n-hexane

> ***No oral RfD has been derived. Has the rationale and justification for not deriving an RfD been transparently described in the documents? Are there additional studies that should be considered in this decision?***

James J. Chen, Ph.D.
The rationale and justification for not deriving an RfD have been transparently described and I am not aware of additional studies.

Lucio G. Costa, Ph.D.
The rationale and justification for not deriving an oral RfD for n-hexane has been well described. I am not aware of any additional studies.

Hugh L. Evans, Ph.D.
Yes.

No.

Doyle G. Graham, M.D., Ph.D.
Rationale and justification well described. No additional studies recommended.

Bernard Weiss, Ph.D.
The absence of an oral RfD is ascribed to the lack of relevant data. In addition, its volatility makes it unlikely that contamination of drinking water derived from surface water would serve as an exposure source even though significant amounts may be found in groundwater.

2) Inhalation reference concentration (RfC) for n-hexane

> ***a) Has the rationale and justification for deriving an RfC been transparently described in the documents? Are there additional studies that should be considered in this decision?***

James J. Chen, Ph.D.
This is a very thorough review of the literature. But, it is difficult to read because several sections repeat the same information; for example, the Altenkirch et al. (1982) and Ichihara et al. (1998) studies discussed on pages 42 and 43 are repeated on page 64. Also, the materials presented are not well organized; the information is simply catalogued from the literature without an attempt to synthesize it.

I am not aware of additional studies that should be considered.

Lucio G. Costa, Ph.D.
The rationale and justification for deriving an inhalation RfC for n-hexane has been well described in the document. However, see comments below on UFs. I am not aware of any additional studies.

Hugh L. Evans, Ph.D.
Yes.

No.

Doyle G. Graham, M.D., Ph.D.
Rationale and justification adequately described. No additional studies recommended.

Bernard Weiss, Ph.D.
The justification is not as clear as the rationale. Presumably, n-hexane warrants a RfC because it is emitted from many sources: industrial plants, gas stations, refineries, consumer products, etc. An expansion of Section 2 would be useful.

The rationale is based on an extensive literature documenting the neurotoxicity of n-hexane. It makes sense, then, to use some index of neurotoxicity, such as MCV, to derive exposure standards. But, particularly in examining the studies reviewed in the document, one is struck by how many other possible endpoints have remained unexplored; for example, subjective responses such as reports of weakness, color vision defects, and other neurobehavioral measures that, given the neurotoxicology literature, might prove more sensitive. Unfortunately, the n-hexane data are so crude that such measures prove unusable for risk assessment.

Some of the background material in 3.2 is confusing. One example is the discrepancy between Table 2 and Table 3, which show marked, unexplained, differences in tissue levels after exposure to 1000 ppm.

Section 4.1.2.2 (Chronic exposure) is a listing of individual studies accompanied by brief descriptions of their contents. It presumably sets the stage for the risk assessment section, but it offers no summary or conclusions about what this listing is supposed to convey.

Similarly, Table 30 (Inhalation studies) lists the studies, but not much guidance on interpretation. The shifting exposure characterizations, from ppm to mg/m^3 are confusing; why the changeovers?

b) The 1990 IRIS assessment for n-hexane used a human occupational exposure study by Sanagi et al. (1980) for the derivation of the RfC. The draft reassessment for n-hexane uses a subchronic rat study by Huang et al. (1989) for the derivation of the RfC. The workers evaluated in the Sanagi

et al. (1980) study had co-exposure to acetone and n-hexane. Data were identified that indicate n-hexane metabolism and n-hexane-induced neurotoxicity are potentiated by co-exposure to acetone. Thus, this study was not selected for the derivation of the RfC in the current assessment.

The rationale supporting selection of the Huang et al. (1989) study versus the Sanagi et al. (1980) study as the principal study in the derivation of the RfC is presented in Sections 5.2.1 and 5.2.4 of the Toxicological Review. Is the Huang et al. (1989) study the most appropriate selection for the principal study (i.e., best study upon which to determine the point of departure)? Has the rationale for this choice been transparently and objectively described? Is the selection of Huang et al. (1989) as the principal study scientifically objective? Is the exclusion of Sanagi et al. (1980) as the principal study based on co-exposure to acetone justified? Should the Huang et al. (1989) study and the Sanagi et al. (1980) study be considered as co-principal studies in the derivation of the RfC?

James J. Chen, Ph.D.
The rationales for exclusion of the Sanagi et al. (1980) study and the selection of the Huang et al. (1989) study as the principal study are transparently described.

The Huang study (1989) is appropriate for dose response modeling and determining POD.

The Mast (1987) reproductive study on SD rats was not selected as the principal study because the range between the NOAEL of 200 ppm and the next highest dose (1000 ppm) is considerable. This reason itself is not sufficient to exclude Mast (1987) from consideration. A large range between the NOAEL and LOAEL does not necessarily result in uncertainty in the dose-response. How well a fitted dose-response function describes the data will be assessed by a goodness-of-fit measure in model assessment.

The BMD modeling was performed on the Mast (1987) reproductive study on SD rats because of a statistically significant reduction in fetal body weight gain in males at 1000 and 5000 ppm doses and a statistically significant increased incidence of reduced skeletal ossification at 5000 ppm. The BMD modeling was not performed on the Mast (1988a) CD-1 mouse study. The descriptions of adverse effects observed on Page 53 indicate high dose effect as well as a dose-response trend for several reproductive/developmental endpoints. The observed effects between the two studies appear comparable. To be consistent, the BMD modeling should be performed on the Mast (1988a) study.

All four studies, Sanagi et al. (1980), Huang et al (1989), and two NTP reproductive/developmental studies (Mast, 1987, 1988a), should be analyzed thoroughly.

Lucio G. Costa, Ph.D.
I would agree with the use of the Huang et al. (1989) study instead of the Sanagi et al. (1980) study for deriving the inhalation RfC for n-hexane, due to a lack of a dose-response and to co-exposure to acetone in the latter study. However, the human study should be not discounted and should be better discussed and integrated with the rat study as they both provide information useful for the risk assessment.

Hugh L. Evans, Ph.D.
Yes.

Yes.

Yes.

Yes, if the mandate here is to review exposure to hexane alone. But a large portion of the people who are exposed to hexane are co-exposed to other solvents or impurities, so the Sanagi study provides a good model for those situations.

Yes. The study by Sanagi et al. 1980 provides useful information about symptoms in humans which cannot be modeled in an animal, e.g., the incidence of headache in exposed workers was double that of controls. The subjects were not evaluated by Sanagi for nausea, a subjective symptom reported by those who work with hexane.

Doyle G. Graham, MD, Ph.D:
The panel agreed, after discussion, that Huang et al. (1989) and Sanagi (1980) should be given equal weight in the derivation of RfC. We have equal confidence in the two studies. We agreed that the confounding presence of acetone in Sanagi would bias the data in the direction of enhanced toxicity and therefore including Sanagi would be a conservative decision. The panel agrees with the document that 58 ppm can be taken as a LOAEL.

Bernard Weiss, Ph.D.
The review document makes clear the deficiencies in both reports. Outcome measures in Sanagi et al were confounded by co-exposure to acetone, which, as documented, may enhance n-hexane neurotoxicity. Huang et al, for all its attention to experimental design, is still an animal study. And, the lowest concentration used by Huang et al, 500 ppm, is ten times the ACGIH TLV. The implicit assumption is that the relationship between exposure and neurotoxic effect is monotonic, even linear. That may not be the case. Ikeda et al (1993), studying acute behavioral effects, found a non-monotonic relationship. Narcosis is a different endpoint than peripheral neuropathy, but the difference may warrant consideration.

The main objection to Sanagi et al is concurrent exposure to other solvents, notably acetone. But if it is chosen as the principal study, the acetone exposure merely plays the role, so to speak, of an additional uncertainty factor. That is, it provides an extra margin of safety in the BMD calculations.

Because using either of these studies as the basis for the risk assessment yields essentially the same value, a reasonable course to pursue would be to choose Sanagi as the principal study, with Huang et al offered as supporting data.

c) Has the most appropriate critical effect (decreased motor nerve conduction velocity in male rats following 12 weeks n-hexane exposure) been selected? Has the rationale and justification for this effect been transparently described? Is the selection of the critical effect scientifically justified?

James J. Chen, Ph.D.
The rationale and justification have been transparently described. The selection is justified.

Lucio G. Costa, Ph.D.
The most critical effect (decreased motor nerve conduction velocity) has been correctly chosen.

Hugh L. Evans, Ph.D.
Yes.

Yes.

Yes.

Doyle G. Graham, M.D., Ph.D.
Given the available studies I agree with selection of MCV decrease as most appropriate critical effect, since determinations of MCV yield quantitative differences suitable for analysis. It must be noted that changes in MCV are slightly less sensitive than would be changes in hindlimb grip strength (see the carbon disulfide studies summarized in Harry, et al. (1998) NeuroToxicology 19: 159-162).

Bernard Weiss, Ph.D.
As noted above, MCV has been adopted as a virtually standard endpoint, so that other endpoints, particularly those based on CNS function, are, in effect, precluded.

d) An RfC has been derived utilizing benchmark dose modeling to define the point of departure. Is benchmark dose modeling the best approach for determining the point of departure? Has the benchmark dose modeling been accurately and transparently described? In the absence of a biological rationale for choosing an appropriate effect level, a point of departure corresponding to a change in the mean equal to one control standard deviation from the control mean has been used. Is this the best approach for determining the effect level? Has the most appropriate model been utilized? Please comment on the model choice (and the values utilized for the model parameters) as well as the approach.

James J. Chen, Ph.D.
Yes, benchmark dose modeling is the best approach for determining the POD.

The BMD was calculated based on a change in the mean equal to one control SD from the control mean. An explanation of this criterion should be included in the text.

Benchmark dose method involves finding a mathematical model to dose response data set. The benchmark dose is defined as the dose that corresponds to a specified change in an adverse response compared to the response in untreated controls or unexposed population. The specified amount of change is used to define the BMR as a measure of increased risk (over background risk). The BMR represents a minimal amount of change in the endpoint that is generally considered to be biologically significant. The benchmark dose (BMD) is the dose that corresponds to the specified BMR. BMR values of 0.01, 0.05, and 0.10 are the most common values that have been considered.

For continuous health effects such as MCV or fetal weight, in the absence of what level of response to consider adverse, a BMR can be set at a change in the mean equal to one standard deviation from the control mean. This definition is equivalent to assuming the tail area of the distribution in control subjects is defined to be abnormal: a response is abnormal if it is below (or above) a cutoff. The size of this tail area constitutes the level of background risk for the continuous effect. In the absence of a clinical definition of an adverse level, a low or high percentile (e.g., the 99^{th} percentile) could be used to define

an abnormal cutoff. Crump (1995) showed the relationship between a change in the mean response, relative to the standard deviation, and the excess risk.
For example, if a value beyond the 98th to 99th percentile of controls is considered to be abnormal, a dose that cause a shift in the average of one standard deviation results in approximately an excess risk of 10% of the animals in the abnormal range. This provides a very simple method for establishing a BMD associated with a risk of approximately 10%. A BMD dose that causes a change in the mean of the continuous response equal to standard deviation is equivalent to defining a BMR of 10% with background risk of 1%.

Appendix B provides a general description of BMD analysis; it also needs to explain connections between the BMD method and BMD Software. What input option and parameters were used to obtain Table B-1 and Output B-2 ?

The BMD was computed based on the Hill model. The exact mathematical equation of the Hill model should be provided in the main text. It also should have discussions on why the Hill model was chosen over other models such as the polynomial or power model. In the 3rd line on page 144, the Hill model for 12 weeks exposure has n = 2, but the fitted model appears to have
n = 1.

Lucio G. Costa, Ph.D.
I do not feel commenting on the choice of model.

Hugh L. Evans, Ph.D.
Yes.

Yes.

Yes.

Yes.

The point of departure was chosen at a very large value (10%), while a point of departure of 5% or 1% would be more in line with current practice and more conserving of health of workers.

Doyle G. Graham, M.D., Ph.D.
Agree with benchmark dose modeling as presented, but I have limited understanding of these analyses.

Bernard Weiss, Ph.D.
BMD modeling is the obvious choice and is consistent with current EPA practice. However, the choice of one SD is not explained clearly. EPA's usual practice is a choice of 10% or 5%.

e) Are the uncertainty factors applied to the point of departure for the derivation of the RfC scientifically justified and transparently and objectively described in the Toxicological Review?

James J. Chen, Ph.D.
A total UF of 1000 was applied to the POD: 10 for intraspecies variation (UFH: human variability); 3 for interspecies differences (UFA); 10 to extrapolate to chronic exposure from data in a less-than lifetime study (UF_S); and 3 to account for database deficiencies (UF_D). The RfC is obtained by BMCL/1000. A UF to account for the extrapolation from a LOAEL to an NOAEL was not applied because BMD modeling was used.

The calculation of RfC implicitly uses the BMDL as an NOAEL. The BMDL of 215 mg/m^3 is calculated based on an excess risk of 10%. The 10% excess risk can not be regarded as an NOAEL. The National Research Council's Subcommittee on Spacecraft Water Exposure Guidelines recommended that the $BMDL_{01}$ be used instead of the NOAEL and the $BMDL_{10}$ be used instead of the LOAEL (NRC, 2000). Also, Farland and Dourson (1992) noted a good correspondence, on average, between the LOAEL and the BMD_{10} for selected chemicals listed on EPA's IRIS. It should provide some justifications for using BMR=10% for the MCV or choosing BMR = 5%.

References:

NRC (2000) Methods for developing spacecraft water exposure guidelines. National Academy Press: Washington, DC.

Farland, W and Dourson, M. (1992). Noncancer health endpoints: approaches to quantitative risk assessment. In: Comparative Environmental Risk Assessment (C.R. Cothern, ed.) Lewis Publishers: Boca Raton, 87-106.

An UF_S of 10 was applied to account for less-than lifetime study. This adjustment appears excessive. A chronic exposure is usually in the range of 90 to 104 weeks. For 16 week exposure that adjustment should not exceed 104/16 about a factor of 7. A factor between 3 to 5 may be sufficient.

Lucio G. Costa, Ph.D.
The uncertainty factors have to better described and justified (see below).

Hugh L. Evans, Ph.D.
I suggest an Uncertainty Factor of 2 be applied if 10% is used as the point of departure.

Doyle G. Graham, MD, Ph D.
Uncertainty factors adequately described, but I disagree with their application, since their application does not give sufficient weight to what is known about the neurobiology of the axon. While I agree that the adjustment of the BMCL to 24 hours/day is justified, I do not see data that would justify a UF of 10 for intraspecies variation. Since the BCML has been based on studies in adult male rats, any variation between young and older individuals would be in the direction of lesser effects in the younger, explained by shorter axons in the CNS and PNS. There are no data to suggest that intraspecies difference exist in absorption, distribution, metabolism, excretion or repair. However, if concerns about genetic polymorphisms are included in this section, then I would supprt an UF of 10 for intraspecies differences. The UF of 3 for animal to human extrapolation is justified and appropriate. The UF of 10 to extrapolate from subchronic exposure is neither justified nor appropriate; in my view, this UF should be no greater than 3, given that 16 weeks is half the time for a newly synthesized neurofilament protein to be transported from the neuronal cell body to the axon terminal in the longest PNS and CNS axons of an

adult rat. There is no justification to extrapolate to a lifetime of the rat, since the lifetime of the target, the neurofilament, is so much shorter. UF of 3 for database deficiencies is probably OK, but concerns about increased susceptibility in the fetus are not supported by the data that show that developmental defects and decreases in MCV occur at about the same concentrations. Thus, I would calculate a total UF of 10 x 3 x 3 x 3 = 270, and would calculate an RfC as 215 mg/m3 divided by 270 = 0.8 mg/m3. Previous SAB calculations based on Sanagi used a total UF of 300; using 58 ppm (= 204 mg/m3) an a LOAEL, 204 divided by 300 = 0.7 mg/m3 for the RfC.

Bernard Weiss, Ph.D.
These choices, although standard practice in EPA, also underscore the deficiencies of the Huang study. The BMCL adjusted for 24-hr exposures is 215 mg/m^3, calculated by halving the 12-hr Huang-based value of 430 mg/m^3. But the same kind of calculation could be used to derive a TLV. That is, adjusting for exposure duration (40 hrs/week versus 84 hrs/week, would yield a value of 451 mg/m^3. Such a value is nine times the TLV. Does such a derivation make sense? The doubts in such reasoning demonstrate the problems in substituting Huang et al for Sanagi et al. Given the end result, it can be argued that both studies, in combination, provide the best basis for calculating a RfC.

The total UF of 1000, given the current data and understanding of n-hexane neurotoxicity, is therefore excessive. The UF of 10 for intraspecies variation is better set to 3. The UF of 10 for subchronic to chronic exposure, given what is known of the toxic mechanism, is more appropriately set to 3 as well.

f) The database for n-hexane is lacking a developmental neurotoxicity study. Given the potential increased susceptibility of the developing fetus to n-hexane-induced toxicity and the increased neurotoxicity in humans and animals following n-hexane exposure, a UF_{DB} of 3 was applied. Has the rationale and justification for the UF_{DB} been transparently described? Is the application of this UF appropriate?

James J. Chen, Ph.D.
A UF_D of 3 was applied to account for database deficiencies. The rationale and justification for the UF have been transparently described. A UF_D of 3 is appropriate.

Lucio G. Costa, Ph.D.
The choice of an UF of 3 for lack of developmental neurotoxicity studies is appropriate, but could be better supported.

Hugh L. Evans, Ph.D.
The UFDB of 3 is justified. See my comments below regarding the wording of the draft in summarizing results of Howd. A more comprehensive statement is needed to tie together what is known about effects on fetus, on the newborn, pre-weaning and young adult. I am not familiar with data on effects on n-hexane on older animals.

Yes.

Doyle G. Graham, M.D., Ph.D.
As outlined above I do not agree that developmental toxicity in itself justifies a UF, but a database UF of 3 is reasonable.

Bernard Weiss, Ph.D.
The reasoning for a UF=3, given the paucity of data, provides a suitable basis for such a value.

3) Carcinogenicity of n-hexane

> ***Under EPA's 1999 Draft Revised Guidelines for Carcinogen Risk Assessment (www.epa.gov/ncea),* data are inadequate for an assessment of the human carcinogenic potential *of n-hexane. Do the available data support this statement? Are there additional studies that should be considered in this decision?***

James J. Chen, Ph.D.
A 2-year carcinogenicity bioassay in mice and rats exposed to a mixture containing n-hexane and various hydrocarbons showed a borderline increased incidence of liver tumors in female mice only. Genotoxicity evidence has been largely negative. Therefore, the available data appears to be inadequate for cancer assessment. I am not aware of additional studies.

However, I disagree with the statement, "The available studies in humans as well as laboratory animals thus far have not demonstrated a carcinogenic effect", in Section 4.61. Based on the summary presented a logical conclusion should be "the available data have not demonstrated a non-carcinogenic effect'' or "the available data are inadequate for cancer risk assessment.''

Lucio G. Costa, Ph.D.
I agree that data are inadequate for an assessment of the human carcinogenic potential of n-hexane.

Hugh L. Evans, Ph.D.
Yes.

No.

Doyle G. Graham, M.D., Ph.D.
I agree data are inadequate to conclude whether or not hexane may be carcinogenic, but I think there is no reason for concern. The Beall et al., 2001 study provides very weak evidence for a cause and effect relationship between hexane exposure and the development of brain tumors. Further, it should be noted that the low pKa of exocyclic amino functions of DNA(<5) would preclude reaction with 2,5-hexanedione to yield pyrrole adducts. Thus the lack of mutagenicity is expected.

Bernard Weiss, Ph.D.
The available literature supports such a conclusion.

Other Comments

James J. Chen, Ph.D.
SECTION 5.2
p101, 2nd para. Both p-values from Mast (1987) are less than .1. Since BMD modeling was not performed on Mast (1988), where do the p-values come from?

p101, 3rd para. Where is Figure 1?

p102. It needs to define $BMCL_{ADJ}$ and $BMCL_{[HFC]}$. The notations $BMCL_{[HFC]}$ and $BMCL_{HFC}$ are inconsistent.

APPENDIX B
p127, 2nd para. Should Mast 1988a be Mast 1987?

p127, 4th para. The last sentence "an extensive sensitivity analysis.. where appropriate''. The results of the extensive analysis have never been mentioned/discussed.

p128, 1st para. The method of using the simple mean and standard deviation across all litters is incorrect in the analysis of developmental effect data. By ignoring the correlation among pups in the same litter will result in overestimating the BMDL. That is, the estimate of BMDL will be higher than the 'true' confidence limit. (The central estimate, BMD, will not have much difference.) A simple (proper) analysis is to use the litter-based analysis. First, compute the mean for each litter within each dose group, and then treat these means as experimental data using Benchmark Dose Software. Of course, these means can be converted to mean and standard deviation. The main difference between the litter-based analysis and the fetal-based analysis is the sample size. In the litter-based analysis the sample size is the number of litters, rather than the number of fetuses (page 137 bottom).

Lucio G. Costa, Ph.D.

p14 A brief mention of acetone should be included when discussing interactions of n-hexane with other chemicals.

p19 Perbellini (typo)

p23 Sanagi et al. (1980). Is the concentration of acetone sufficient to affect n-hexane? Are there any information on this issue?

p25 A summary Table relating exposure levels and effects in humans would be useful.

p40 Why use the term prechronic instead of subchronic?

p44 Need more details. NSE and ?β-S-100 are usually used as markers of neurons and astrocytes, respectively. The same decrease of 75% of β-S-100 at all dose levels is somewhat strange.

p52 Study by Mast (1987) is not very useful

p67 The study by Cardona et al. (1996) does not appear to be relevant. The study of Ladefoged et al (1989) is relevant; however, the interaction is not explainable by induction of CYP2E1.

p80 The sentence "the mechanism of neurotoxicity of n-hexane following oral exposure is not well understood" is a bit misleading, given the work by Graham et al.

p82 The study of Ono et al. (1982) established a LOAEL of 200 ppm. It should be better discussed in relationship to other NOAEL and LOAEL values.

p92 Children may actually be less sensitive to n-hexane because of lower CYP2E1 levels

p92 Ethanol, as an inducer of CYP2E1, would be expected to increase the toxicity of n-hexane. Ladefoged et al. (1989) found no interactions, but they looked at 2,5 hexanedione rather than n-hexane.
p96 Define better "relatively low doses".

p102 UF of 10 for human variability should be based not on developmental considerations but rather on possible genetic polymorphisms

p103 UF of 10 for subchronic to chronic is not well justified and could be reduced to 3 (see additional considerations below)

p103 UF of 3 for data base deficiency needs to be better discussed

p104 The total UF, as presented is 900 (3x10x10x3), not 1000. Thus, RfC should be 215/900 = 0.24 mg/m^3. If subchronic to chronic UF is 3 (instead of 10), then total UF = 270 and RfC would be 215/270 = 0.8 mg/m^3.

The effects seen in subchronic studies are the same as in chronic studies. Evidence is not presented of cumulative damage or of dose-rate effects, such as those seen with acrylamide.

Hugh L. Evans, Ph.D.
Many of the findings reviewed here are the result of selected data from only a single published study. Many key findings should be replicated, and the need for those replications should be publicized.

p25, next to last sentence. It is not justified to state " ..the degree to which these changes represent impairment of neurological function is uncertain, …" when summarizing the results of Sanagai et al., 1980 in Tables 9 and 10. The fact that 2 of 8 endpoints were significantly affected by exposure (Table 9) or 2 of 6 (Table 10) typifies near-threshold effects that seem to be more readily accepted in other sections of this document. I suggest deletion of the sentence implying uncertainty.

p37, next to last paragraph. "cement coasting" is probably meant to be "coating" as that term is used in the final paragraph of this page.

p38, last full paragraph. This report by Smith & Albers 1997 is given more emphasis than it deserves. There was only one subject, so line four should change "subjects" to "subject." The draft statement does not make clear that this subject had a history of exposure to several recreational drugs as well as to several non-hexane constituents of the glue.

p68, top paragraph. "significantly correlation" should be "correlated."

p68, second paragraph. "Body weigh gain and water consumption was …" should be "were."

p69, first paragraph. "One a week" should be "once".

p80, line 3 “injected subcutaneously” this study does not belong under heading “4.5.1 Oral Exposure.”

p80, paragraph 3. Summary statement should say explicitly “there are no human data.”

pp82-83, The term “compound-related” appears 4 times here, but nowhere else in the draft. The reader is not clear what is meant, as n-hexane usually is not described as a “compound.” Suggest delete term or replace it with a more concise term, e.g., “hexane-related” or “dose-related” if that is what is intended.

pp84-85, What is the policy of citing abstracts that are not available in Medline or archival literature? For example, Soiefer et al., 1991 and Duffy et al., 1991, and several similar abstracts are listed in the References. On the one hand, you are doing a service to dredge up every scrap of information that can be found. On the other hand, with this document’s heavy emphasis upon peer review, these abstracts have had no peer review nor do they state sufficient information about methods and analysis of data to permit an independent appraisal of the study’s value. Other cited works that have not been published in archival scientific literature exist in the form of technical reports, with more information, submitted to EPA or another organization, that can be reviewed in depth.

p87, Table 30. Make this more user friendly. What rationale, if any, was used in deciding which sequence to list the references? Couldn’t they be ordered in some meaningful way?

p90, first paragraph. The wording “was inconclusive” does not convey the correct information because it may cause the reader to assume, incorrectly, that there were no statistically significant findings. Suggest saying instead that “because the workers also were exposed to other potential carcinogens, we cannot attribute the elevated odds of cancer to hexane alone.”

p91, second full paragraph. The wording, as in the above comment, “reduce the significance of the results” implies that the results are not significant. In fact, they may be highly significant for the common situation of co-exposure to several solvents. Suggest change in wording in accord to my comment above, such as “because of co-exposure to several other chemicals, results do not permit a conclusion about hexane alone.”

p92, first paragraph under 4.7.1. Related to my response to the Charge section 2 f. The wording “weanling rats were more resistant” may create an incorrect impression in the reader that the weanlings were scarcely affected. This does not capture the important essence of this study by Howd et al., 1983, which showed statistically and toxicologically significant effects in weanling rats. Young adult rats showed similar effects, but perhaps of greater magnitude or earlier onset. Differences between the two age groups should be reported, and are discussed intelligently in the draft, but the differences are not so profound, and are of the type that should be replicated if the age-difference were to become a key point in setting policy.

p103, first paragraph, same comment as above on interpretation of data from Howd et al. The weanling rats showed effects that would be devastating to a human. To conclude that the young rats were “less sensitive” seems to deflect the reader’s attention to an age-difference that seems less than profound and deserves to be replicated if it were to be the basis of a major decision.

p104, top. The document should be more user friendly in reporting the calculations. It should also report the result of the calculation in the more familiar terms (e.g., ppm) that are used elsewhere in this document and in the scientific literature.

Doyle G. Graham, M.D., Ph.D.

pp8 and 14, To the best of my knowledge, furans do not form *in vivo*, but are generated during acid hydrolysis of urine prior to GC/MS.

pp9 and 10, It would be my guess that acid hydrolysis also releases 2,5-hexanedione that has been bound to protein amino functions through a reversible imine bond. I do not know about the contribution of 4,5-dihydroxy-2-hexanone.

p17, Can dimethylfuran be hydrolyzed to 2,5-hexanedione? Probably only under acidic conditions (not *in vivo*).

p27, partial paragraph ending with (CNS): Add: "Indeed, studies in rats (Schaumburg and Spencer, 1976) showed that neurofilament-filled axonal swellings developed in the sub-terminal regions of the longest ascending and descending axons in the spinal cord concomitant with involvement of the longest axons in the peripheral nervous system."
[Ref: Schaumburg, HH; Spencer PS. (1976) Degeneration in central and peripheral nervous system produced by pure n-hexane – An experimental study. Brain 99: 183-192.]

p31, end of second complete paragraph: Substitute "electromyographic" for "myographic."

p36, next to last line: Substitute "polyneuropathy" for "polyneuritis" (the latter term is archaic and erroneously suggests an inflammatory etiology).

p41, last paragraph, second line: insert "oil" after "olive".

p42, last paragraph of section 4.2.1.1 appearing as a partial paragraph on p.42. Suggest adding, "In the absence of histological studies the significance of minor MCV changes after exposure to 2- and 3-methylpentane and methylcyclopentane cannot be evaluated; the generalization now embraced is that for an alkane to be neurotoxic it must be metabolized to a γ-diketone, not that it is a "hexacarbon" (Graham, et al., 1995).

p43, first full paragraph, line 12: change "(distal and proximal)" to" (distal plus proximal)"

p43, first full paragraph, line 14: change "myelin sheath" to "myelinated".

p43, last full paragraph, change last sentence to "Additionally, employing only H&E stained sections of paraffin-embedded nerve, no histopathological effects on the peripheral nerves were observed at term (14 weeks)."

p50, first paragraph, line 5: change "microscope" to "microscopes"

p.57, fourth paragraph, line 3: change "possibly correlated" to "accompanied by" (the former suggests a pathogenetic relationship)

p67, last line: change “various forms of” to “concentrations of free and total (acid hydrolyzed)”

p70, last 3 lines: change “first on the proximal sides of the paranodes” to “first proximal to nodes of Ranvier”.

p71, second full paragraph, last sentence: Omit “(dying back)” [Note that the concept of “dying back” originated by John Cavanagh postulated that toxicants injured the neuron cell body causing the neuron to save itself by dying back its most distal axon.]

p72, second paragraph, first sentence: change “are not completely understood” to “have been studied extensively.”
p72, second paragraph, second sentence: change “pyrroles” to “pyrrole adducts that then undergo oxidation, leading to protein crosslinking.”

p72, second paragraph, last sentence: change “peptides” to “proteins”

p.72, last paragraph, last line: after “2,4-hexanedione” insert “, a β-diketone that cannot react with protein amino functions to form pyrrole adducts,”

p73, first full paragraph: replace last 2 sentences with “This study confirms that only diketones with γ-spacing are capable of forming pyrrole adducts, a necessary step in the neurotoxicity of alkanes.”

p73, third line from bottom: Omit “Although the sequence of critical events remains uncertain,”

p74, first line: change to “cyclization to form pyrrole adducts that then undergo oxidation to electrophiles that react with protein nucleophiles to result in covalent crosslinking of derivatized proteins to form higher molecular weight protein aggregates.”

p74, add paragraph between Anthony 1983b and Boekelheide 1987:

> Genter, et al. (1987) separated the *d,l* from the *meso* diastereomers of 3,4-dimethyl-2,5-hexanedione, both of which form identical tetramethylpyrrolyl adducts in the reaction with protein amino functions. The *d,l* diastereomer both formed pyrroles more rapidly and was more neurotoxic than the *meso* diastereomer, strongly supporting the concept that pyrrole adduct formation is a necessary step in the pathogenesis of γ-diketone neurotoxicity. Rosenberg, et al. (1987) showed that the axonal swellings that followed intoxication with the *d,l* diastereomer were demonstrably more proximal than those produced by the *meso* diastereomer, showing that the rate of protein crosslinking determines the proximo-distal location of the axonal swellings.

[Refs: Genter MB; Szak·l-Quin G; Anthony DC; Graham DG. (1987)Evidence that pyrrole formation is a pathogenetic step in γ-diketone neuropathy. Toxicol. Appl. Pharmacol. 87:351-362.
Rosenberg CK; Genter MB; Szakal-Quin G; Anthony DC; Graham DG. (1987) *d,l* Versus *meso* 3,4-dimethyl-2,5-hexanedione: A morphometric study of the proximo-distal distribution of axonal swellings in the anterior root of the rat. Toxicol. Appl. Pharmacol. 87:363-373.]

p75, second paragraph: first line: change “diketone” to “γ-diketone”

p75, second paragraph: beginning on line 12 with "The authors…" replace the next 2 sentences with "The authors postulated that the electron-withdrawing acetyl group on the pyrrole formed by 3-acetyl-2,5-hexanedione rendered the pyrrole ring less susceptible to oxidation. In the absence of oxidation of the pyrrole ring, crosslinking of proteins could not occur." Then continue with "This hypothesis.." In the last sentence add "both" between "that" and "pyrrole". As a last sentence in this paragraph, add "The observation that neurological deficit continues to worsen for several weeks after cessation of exposure of humans to n-hexane can be explained by the continuing oxidation of pyrrole rings and neurofilament crosslinking."

p76, add the following paragraph to the end of section 4.4.4:

> An observation that has been made consistently between species, between adult and immature members of the same species, and within individual humans and animals, is that longer axons in the PNS and CNS are more vulnerable than shorter axons to the toxic effects of n-hexane and its metabolites. The axonal swellings that initially occur proximal to nodes of Ranvier in the most distal internodes of the longest axons are filled with disorganized masses of neurofilaments. Thus, Graham et al. (1995) have postulated that during repeated exposures to n-hexane the resulting metabolism to 2,5-hexanedione results in progressive derivatization of protein lysyl amino functions to form pyrrolyl adducts; oxidation of the pyrrole rings to electrophiles leads to increasing levels of crosslinking of neurofilaments during the proximo-distal transport of axoplasm. Further, the observations by Cavanagh and Bennetts (1981) suggest that the constrictions of axonal diameter at nodes of Ranvier contribute to the formation of axonal swellings at these locations by presenting obstructions to the transport of the growing masses of neurofilaments; additionally, they observed that non-obstructing masses of neurofilaments could be successfully transported to the synapse for proteolysis. Since the rate of neurofilament transport is 1 mm/day (Griffin, et al., 1984), axonal length can be seen to determine the period of time during which sufficient neurofilament crosslinking must occur to produce the threshold masses necessary to occlude transport and result in axonal swellings, secondary myelin retraction and demyelination, and distal axonal degeneration.

[Refs: Cavanagh JB; Bennetts BJ. (1981) On the pattern of changes in the rat nervous system produced by 2,5-hexanediol. A topographical study by light microscopy. Brain 104: 297-318.

Griffin JW; Anthony DC; Fahnestock KE; Hoffman PN; Graham DG. (1984) 3,4-Dimethyl-2,5-hexanedione impairs the axonal transport of neurofilament proteins. J. Neurosci. 4:1516-1526.]

p79, third full paragraph, first line: change "suggesting" to "showing"

p79, fourth full paragraph, last sentence: change "may have occurred" to "had occurred"

p80, last paragraph: in second sentence change "may be associated" to "is". End last sentence with "… in the posterior tibial nerve after cessation of exposure."

p82, first full paragraph: change last sentence to "Ono (1982) established a LOAEL of 200 ppm for histopathological effects characterized by axonal swellings and degeneration of myelinated axons in Wistar rats subchronically exposed to 200 and 500 ppm n-hexane."

p85, second full paragraph: change third sentence to read, "… sequence of events including accumulations of neurofilament-filled axonal swellings and secondary demyelination, that could lead to a decrease …"

p85, third full paragraph:

sentence one: change "suggest" to "show"

sentence two: insert "secondary" before "myelin"

sentence four: Change to "*In vivo* and *in vitro* studies indicate that the mode of action of 2,5-hexanedione includes covalent crosslinking of neurofilament proteins in peripheral nerve and spinal cord."

sentence five: "Studies show that 2,5-hexanedione reacts with …"

sentence seven: change "with the" to "of"

sentence eight: change to "Formation of pyrrole adducts, followed by oxidation of the pyrrole rings and crosslinking of neurfilaments, has been …"

p86, line 3: change "1,2-diacetylethane" to "1,2-diacetylbenzene"

p86, end paragraph with this sentence: "Further, studies detailing the neurotoxicity of the *d,l* and *meso* diastereomers of 3,4-dimethyl-2,5-hexanedione (Genter, 1987) and of 3-acetyl-2,5-hexanedione (Genter St. Clair, 1988) demonstrate that both pyrrole formation and subsequent oxidation of the pyrrole rings to result in neurofilament crosslinking are necessary steps in the pathogenesis of n-hexane neurotoxicity."

p97: last paragraph, line 9: change "myelin sheath" to "myelinated"

p106, last paragraph: change "peripheral nerve filaments" to "neurofilaments"

p112, Cavender ref: change "Fandom" to "Fundam"